SANDIA REPORT

SAND2005-0485
Unlimited Release
Printed February 2005

Nanofluidic Devices for Rapid Detection of Virus Particles

Paul L. Gourley, Judy K. Hendricks, and Anthony E. McDonald

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

Sandia is a multiprogram laboratory operated by Sandia Corporation,
a Lockheed Martin Company, for the United States Department of Energy's
National Nuclear Security Administration under Contract DE-AC04-94AL85000.

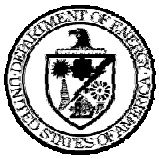
Issued by Sandia National Laboratories, operated for the United States Department of Energy by Sandia Corporation.

Available to DOE and DOE contractors from
U.S. Department of Energy
Office of Scientific and Technical Information
P.O. Box 62
Oak Ridge, TN 37831

Telephone: (865)576-8401
Facsimile: (865)576-5728
E-Mail: reports@adonis.osti.gov
Online ordering: http://www.osti.gov/bridge

Available to the public from
U.S. Department of Commerce
National Technical Information Service
5285 Port Royal Rd
Springfield, VA 22161

Telephone: (800)553-6847
Facsimile: (703)605-6900
E-Mail: orders@ntis.fedworld.gov
Online order: http://www.ntis.gov/help/ordermethods.asp?loc=7-4-0#online



SAND2005-0485
Unlimited Release
Printed January 2005

Nanofluidic Devices for Rapid Detection of Virus Particles

Paul L. Gourley and Anthony McDonald
Biomolecular Materials and Interfaces Department
Sandia National Laboratories
P.O. Box 5800
Albuquerque, NM 87185-1413

Judy K. Hendricks
Contractor

Abstract

Technologies that could quickly detect and identify virus particles would play a critical role in fighting bioterrorism and help to contain the rapid spread of disease. Of special interest is the ability to detect the presence and movement of virions without chemically modifying them by attaching molecular probes. This would be useful for rapid detection of pathogens in food or water supplies without the use of expensive chemical reagents. Such detection requires new devices to quickly screen for the presence of tiny pathogens. To develop such a device, we fabricated nanochannels to transport virus particles through ultrashort laser cavities and measured the lasing output as a sensor for virions. To understand this transduction mechanism, we also investigated light scattering from virions, both to determine the magnitude of the scattered signal and to use it to investigate the motion of virions.

This page left intentionally blank

Contents

This page left intentionally blank

Introduction

Technologies that could quickly detect and identify virus particles would play a critical role in fighting bioterrorism and help to contain the rapid spread of disease. Of special interest is the ability to detect the presence and movement of virions without chemically modifying them by attaching molecular probes. This would be useful for rapid detection of pathogens in food or water supplies without the use of expensive chemical reagents. Such detection requires new devices to quickly screen for the presence of tiny pathogens. To develop such a device, we fabricated nanochannels to transport virus particles through ultrashort laser cavities and measured the lasing output as a sensor for virions. To understand this transduction mechanism, we also investigated light scattering from virions, both to determine the magnitude of the scattered signal and to use it to investigate the motion of virions.

Fabrication of micro- and nanoscale channels

We designed mask set for nanometer scale channels to be fabricated by lithography for transporting submicron organelles, pathogens, and macromolecules through laser cavities. We performed inductively coupled etching experiments on glass and quartz substrate materials. We determined the limits of mask selectivity, etch chemistry, and etch rates for 10 to 1000 nm channel structures. By comparing Ar+ milling and CHF_3 etching condtions, we were able to determine conditions for optically smooth surfaces. Using these conditions, we fabricated nanochannels to transport virus particles through ultrashort laser cavities.

Flow Experiments

These channels are about 100 times smaller than previous laser microcavities used for studying biological cells. The small virus size (~20 to 100 nm) requires new spectroscopic techniques for detection and differentiation. We investigated intracavity quantum photonics, using direct pathogen-supported lasing modes and indirect modulation of the modal noise in stimulated emission from the laser cavity. We used Bacillus megaterium virus since it is relatively large ~100 nm. The virions were flowed through the laser cavity. The bacteria did modulate the noise spectrum of the cavity modes as measured with a spectrum analyzer in the frequency range 0 to 100 KHz, but no evidence of directly supported lasing modes was observed. Thus, flow experiments using bacterial virions results in observable modulation of the noise spectrum of the bare cavity modes, but rules out the possibility of direct virion-supported lasing modes.

Light Scattering

We also performed coherent and incoherent light scattering measurements to determine the magnitude of the scattered intensity from virions and other non-biological submicron particles. The measurements were performed in flow channels, cuvettes, and with high resolution laser scanning confocal microscopy. These experiments revealed several

experimental difficulties associated with light scattering from solutions of small particle. A major concern is the prescence of interfering light scattering from the solution bounding material and surfaces and contaminating particle present in solution. There is a further difficulty of interpreting the scattering distribution and relating it to the particulates of interest.

It is helpful to understand the theoretical light scattering distribution as a function of particle size. We carried out finite difference time domain calculations to determine the transition from Raleigh to Mie scattering. Those calculation show that the transition from Raleigh to Mie scattering from small particles occurs near 200 nm. Since the viruses exist in the Raleigh limit where the scattering is more isotropic, they act primarily as a loss mechanism to modulate the cavity lasing modes as observed in the experiments. The scattering loss is predicted to scale quadratically with virus size and should be tested in future experiments.

Motion of virions

It is of interest to detect the presence and movement of virions without chemically modifying them by attaching molecular probes. This would be of use for rapid detection of pathogens in food or water supplies without the use of expensive chemical reagents. We investigated the use of light scattering to examine its sensitivity in detecting a single virion. To accomplish this task, we observed light scattering from virions on glass slides using an ultra dark field method with an oil-immersion microscope. The light scattering images were observed visually using a broadband light incoherent light source and a laser scanning source. It was possible to directly observe scattered light from virions and their Brownian movement with these techniques, despite the fact that the virions are nearly five to ten times smaller than the wavelength of light used. Theoretical estimates of the magnitude of light scattered from a single virion confirm that this is possible using conventional tungsten lamp sources and optics. However, the virion size is below the resolving power of the microscope and no information about the virion size and shape can be obtained by imaging.

By using laser scanning microscopy, it was possible to record movies of the virion motion and indirectly determine the size of the virion by kinetic studies. This was accomplished by using a bright laser source focused to the diffraction limit and scanned across a selected field of view (~ 100 square microns). The frame rate for a small field of view could be as short as tens of milliseconds, rapid enough to observe virions steps within the field of view. Using a dark field imaging technique with sensitive photomultiplier detection enabled us to record high signal to noise movies of the virion motion.

An experiment was designed to detect the Brownian motion of two different virions to ascertain their mobility. We used a large virion Bacillus megaterium about 100 nm in diameter) and a small virion (Pseudoalteromonas espejiana phage about 60 nm in diameter). From the framing images (taken very 34 ms) we constructed histograms of virion step size. The histograms of step size frequency revealed a Poissonian-like

distribution with about 410 nm average step size for the Bacillus megaterium virus. The smaller Pseudoalteromonas espejiana phage had a larger 500nm step size and exhibited a a more Gaussian distributions. These data demonstrate that virion motion can be used to help differentiate virions.

In the analysis of these data we use statistical thermodynamics of Brownian motion. Stoke's law states that the friction constant α is given by $\alpha=6\pi\eta a$ where **a** is the radius of a macroscopic particle moving in a liquid of viscosity η. If we look at magnitude of the change in position **x** of a virus in water solution during the time between frames of our experiment as that of a diffusing particle executing a random walk so that $\langle x^2 \rangle$ is proportional to time **t** then $\langle x^2 \rangle = t(kT)/(3\pi\eta a)$. Using a room temperature value of **kT** to be 0.025 eV and the viscosity of water, $\eta = 0.0089$ poise we can calculate a value for the radius of "spherical" virus based on the average magnitude of displacement observed frame-to-frame. For one virus population the average displacement magnitude was 0.50 μm which leads to a calculated particle radius of 65 nm. For a second virus population the average observed displacement was 0.41 μm yielding a calculated particle radius of 96 nm. The calculated particle sizes are approximately twice that of the sizes published by the supplier for these virus samples. One possible explanation for this discrepancy is the fact that the formula used to calculate the particle sizes is for macroscopic rather than nanoscale particles. The frictional viscosity forces may vary at this scale. Additionally these virus particles are not spherical as is assumed in the calculation.

Summary

The results show that virions can be detected by light scattering. The light scattering can also be used to determine motion of virions. Intracavity spectroscopy showed that the virions influenced the lasing output, but more work is needed to improve the sensitivity of the technique.

Distribution

1	MS 0123	Donna L. Chavez, 1011
10	MS 1413	Paul Gourley, 1116
1	MS 1413	Judy Hendricks, 1116
1	MS 1413	Anthony McDonald, 1116
1	MS 9018	Central Technical Files, 8945-1
2	MS 0899	Technical Library, 9616